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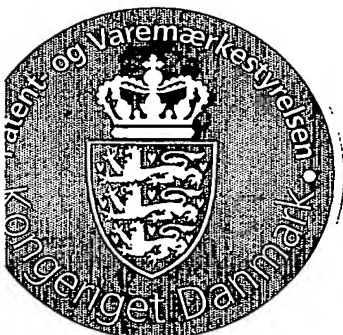
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Title: Use of a secretagogue for the treatment of lipodystrophy

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17 August 2004

Pia Høybye-Olsen



**PATENT- OG VAREMÆRKESTYRELSEN**

05 AUG. 2003

**Use of a secretagogue for the treatment of lipodystrophy****Modtaget**

All patent and non-patent references cited in the application, or in the present application, are also hereby incorporated by reference in their entirety.

5

**Field of invention**

The present invention relates to the use of a secretagogue, such as a ghrelin-like compound for the production of medicament for the treatment or prevention of lipodystrophy as well as to a method of treating or preventing lipodystrophy in an individual in need thereof by administering a secretagogue, such as a ghrelin-like compound.

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**Background of invention**

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Lipodystrophic syndromes encompass a heterogeneous group of rare disorders characterized by partial or generalized loss of adipose tissue depots [Am J Med 2000 108, 143-152]. There are several different types of lipodystrophies and the degree of fat loss may vary from very small depressed areas to near complete absence of adipose tissue. Some patients may have only cosmetic problems while others may also have severe metabolic complications such as dyslipidemia, hepatic steatosis, and severe insulin resistance [Trend Endo Meta 200011:410-416]. These disorders can either be inherited (familial or genetic lipodystrophies) or can occur secondary to various types of illnesses or drugs (acquired lipodystrophies).

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Inherited lipodystrophies are caused by mutations (alterations or blips) in a gene. Several genes responsible for different types of inherited lipodystrophies have been identified. These include AGPAT2 (1-acylglycerol-3-phosphate-O-acyltransferase 2), BSCL2 (Berardinelli-Seip congenital lipodystrophy 2) in Congenital Generalized Lipodystrophy (CGL), Lamin A/ C (LMNA) gene in Familial Partial Lipodystrophy Dunnigan variety (Familial partial lipodystrophy), and PPARG (peroxisome proliferator-activated receptor gamma) gene in familial partial lipodystrophy. Several other candidate genes are currently under investigation for other varieties of inherited lipodystrophies.

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Acquired lipodystrophies are; HAART/HIV-induced Lipodystrophy in HIV-infected patients (LD-HIV), Acquired Generalized Lipodystrophy (AGL), Acquired Partial Lipodystrophy (APL) and localized lipodystrophy. Acquired lipodystrophies do not  
5 have a direct genetic basis. Rather, many mechanisms may be involved. One such mechanism may be an autoimmune response that destroys normal fat cells.

HAART/HIV-induced Lipodystrophy has become the most common acquired form of generalized Lipodystrophy. The overall incidence of these physical abnormalities is  
10 about 50% after 12-18 month of therapy with protease inhibitors. The difference between the present reports range from 18% to 83% percent due to confounding factors such as type and duration of the retroviral therapy. It has been suggested that the lipodystrophy syndrome associated with protease inhibitors may be due to partial analogy between lipid and adipocyte regulatory proteins and the catalytic site  
15 of HIV-1 protease to which the protease inhibitors bind (Carr et al lancet 1998, 351 1881-83).

Localized lipodystrophies are defined as a localized loss of subcutaneous fat from small areas or from parts of a limb. There may be single or multiple lesions. It is  
20 characterized by depressed areas corresponding to the loss of subcutaneous fat. In some cases, it may be associated with tender, painful nodules in the skin. Usually, it occurs in diabetic patients at the site of insulin injections. In some patients, at loss occurs from areas where pressure is applied frequently. For example, pressing thigh against a make-up table.

25

The pathogenesis of lipodystrophy is largely unknown. However accumulating evidences point at mitochondrial defect as central for an increased induction of apoptosis in the adipocytes (6). Several proteins encoded by HIV-1 trigger apoptosis by inducing permeabilization of the mitochondrial membrane. Several nucleoside ana-  
30 logs used clinically in the treatment of HIV-1 inhibit the replication of mitochondrial DNA (mtDNA) and/or increase the frequency of mtDNA mutations. Both of these factors may cause severe mitochondriopathy and contribute to lipodystrophy.

The metabolic consequences of lipodystrophy are highly important for the general health and the survival. The fact that insulin resistance and the consequent progression to diabetes can result from either obesity or lipodystrophy reflects the crucial role of adipose tissue in carbohydrate and lipid metabolism. In the absence of adequate adipocyte capacity, excess calories cannot be diverted to their normal storage depot; instead they accumulate as increased triglyceride stores in liver, in skeletal and cardiac muscle, and in the pancreatic  $\beta$  cell. This extra-adipose lipid accumulation, through as-yet unclear means, is associated with impaired insulin action and, often, diabetes.

In addition to their passive role as storage depots, normal adipocytes secrete a number of peptides ("adipokines") that may influence insulin sensitivity and/or energy balance (Kahn JCI and TEM 2002). These include potential insulin sensitizers, such as leptin and Acrp30 (also known as adiponectin), and insulin antagonists, including  $\text{TNF-}\alpha$ , IL-6, and possibly resistin. The insulin resistance of lipodystrophy may therefore be the result of disturbed lipid fluxes and/or abnormalities of adipokine secretion.

Therapy with rhGH has been reported to cause reduction in the size of 'buffalo hump' and truncal fat in a small number of patients. However, fat loss and lipid abnormalities did not improve and blood glucose control worsened.

Ghrelin is a bioactive peptide which originally was described to be involved in the control of GH secretion but later found to be a major regulator of appetite, food intake and energy homeostasis (1;2). As many other bioactive peptides ghrelin probably act both as a hormone, a paracrine substance and as a neurotransmitter.

The story of ghrelin, its receptor and synthetic compounds acting through this receptor unraveled in a unique "reverse" order. In the eighties a synthetic hexapeptide from a series of opioid-like peptides was found to be able to release growth hormone (GH) from isolated pituitary cells (3). Since this action was independent of the growth hormone releasing hormone (GHRH) receptor, several pharmaceutical companies embarked upon drug discovery projects based on this hexa-peptide GH secretagogue (GHS) and its putative receptor. Several series of potent and efficient peptide as well as non-peptide GH secretagogues were consequently described in the mid nineties (4-6). However, first several years later was the receptor through which these artificial GH secretagogues acted eventually cloned and shown to be a

member of the 7TM G protein coupled receptor family (7;8). But, first in 1999 was the endogenous ligand for this receptor the hormone ghrelin finally discovered (9). The main site for ghrelin production is the stomach, where the peptide is found in classical endocrine cells in the gastric mucosa.

5

### Summary of Invention

The present invention relates to the use of a secretagogue, in particular a ghrelin-like compound, including human ghrelin, in the treatment or prophylaxis of lipodystrophy, whether acquired or congenital, local or generalized.

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In a preferred embodiment the invention relates to the use of a ghrelin-like compound for the preparation of a medicament for the treatment or prophylaxis of lipodystrophy, wherein the ghrelin-like compound comprises a structure defined by formula I

15

$Z^1 - (X^1)_m - (X^2) - (X^3)_n - Z^2$ , wherein

$Z^1$  is an optionally present protecting group

20

each  $X^1$  is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

25

$X^2$  is any amino acid selected from naturally occurring and synthetic occurring amino acids, said amino acid being modified with a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

30

each  $X^3$  is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

wherein one or more of  $X^1$  and  $X^3$  optionally may be modified by a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

$Z^2$  is an optionally present protecting group,

35

m is an integer in the range of from 1-10

n is 0 or an integer in the range of from 1-35.

5 Accordingly, the term "ghrelin-like compound" includes the naturally occurring 28 aa human ghrelin, the amino acid of which is shown in SEQ ID NO: 1, as well as the naturally occurring 27 aa human ghrelin, the amino acid of which is shown in SEQ ID NO: 2. Thus, the present invention relates to the use of ghrelin or a peptide homologous thereto. Ghrelin is described by Kojima in Nature (1999), vol. 402,656-10 660.

The present invention significantly lowers the risk of developing lipodystrophy, independent of the cause, such as independent of the particular therapeutic cause of, or therapeutic factor contributing to, the lipodystrophy. Hence, the present 15 invention also extends to treatment of patients with HIV/AIDS by means of antiretroviral agents. For convenience, the term "antiretroviral therapy" is used herein to refer to the treatment of HIV/AIDS in patients by means of antiretroviral agents generally including combinations of such agents as is typically the case.

20

## **Detailed description of the invention**

### **Definitions**

25 **Affinity:** the strength of binding between receptors and their ligands, for example between an antibody and its antigen.

**Amino Acid Residue:** An amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The amino acid residues described herein are 30 preferably in the "L" isomeric form. However, the amino acid encompasses every amino acid such as L-amino acid, D-amino acid, alpha -amino acid, beta -amino acid, gamma -amino acid, natural amino acid and synthetic amino acid or the like as long as the desired functional property is retained by the polypeptide. NH<sub>2</sub> refers to the free amino group present at the amino terminus of a polypeptide. COOH re- 35 fers to the free carboxy group present at the carboxy terminus of a polypeptide. In

keeping with standard polypeptide, abbreviations for amino acid residues are shown in the following Table of Correspondence:

TABLE OF CORRESPONDENCE

5	SYMBOL		
	1-Letter	3-Letter	AMINO ACID
	Y	Tyr	tyrosine
	G	Gly	glycine
	F	Phe	phenylalanine
10	M	Met	methionine
	A	Ala	alanine
	S	Ser	serine
	I	Ile	isoleucine
	L	Leu	leucine
15	T	Thr	threonine
	V	Val	valine
	P	Pro	proline
	K	Lys	lysine
	H	His	histidine
20	Q	Gln	glutamine
	E	Glu	glutamic acid
	Z	Glx	Glu and/or Gln
	W	Trp	tryptophan
	R	Arg	arginine
25	D	Asp	aspartic acid
	N	Asn	asparagine
	B	Asx	Asn and/or Asp
	C	Cys	cysteine
30	X	Xaa	Unknown or other

It should be noted that all amino acid residue sequences represented herein by formulae have a left-to-right orientation in the conventional direction of amino terminus to carboxy terminus. In addition, the phrase "amino acid residue" is broadly defined to include the amino acids listed in the Table of Correspondence and modified and non-naturally occurring amino acids. Furthermore, it should be noted that a dash at

the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino acid residues or a covalent bond to an amino-terminal group such as  $\text{NH}_2$  or acetyl or to a carboxy-terminal group such as  $\text{COOH}$ .

5

BMI measures your height/weight ratio. It is determined by calculating weight in kilograms divided by the square of height in meters. The BMI "normal" range is 19-22.

10

Concentration equivalent: A concentration equivalent is an Equivalent dosage being defined as the dosage of a ghrelin-like compound having in vitro and/or in vivo the same response as evaluated from a dosage-response curve as wild-type ghrelin.

15

Dissociation constant,  $K_d$ : a measure to describe the strength of binding (or affinity or avidity) between receptors and their ligands, for example an antibody and its antigen. The smaller  $K_d$  the stronger binding.

20

Fusion Polypeptide: A polypeptide comprised of at least two polypeptides and a linking sequence to operatively link the two polypeptides into one continuous polypeptide. The two polypeptides linked in a fusion polypeptide are typically derived from two independent sources, and therefore a fusion polypeptide comprises two linked polypeptides not normally found linked in nature.

25

Ghrelin: a polypeptide as described in Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K 1999 Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402:656-660. Human 28 aa ghrelin has the amino acid of SEQ ID NO: 1.

30

GHS: growth hormone secretagogue

GHS-R 1a: the receptor for GHS. GHS-R 1a is also denoted GHS 1a.

HAART: Highly active antiretroviral therapy.



**Immunologically distinct:** The phrase immunologically distinct refers to the ability to distinguish between two polypeptides on the ability of an antibody to specifically bind one of the polypeptides and not specifically bind the other polypeptide.

5      **Individual:** A living animal or human in need of susceptible to a condition, in particular a cachectic condition as defined herein. In preferred embodiments, the subject is a mammal, including humans and non-human mammals such as dogs, cats, pigs, cows, sheep, goats, horses, rats, and mice. In the most preferred embodiment, the subject is a human.

10

**Isolated:** is used to describe the various ghrelin-like compounds, polypeptides and nucleotides disclosed herein, that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified.

15

**Modified amino acid:** an amino acid wherein an arbitrary group thereof is chemically modified. In particular, a modified amino acid chemically modified at the alpha - carbon atom in an alpha -amino acid is preferable.

20

**Monoclonal Antibody:** The phrase monoclonal antibody in its various grammatical forms refers to a population of antibody molecules that contains only one species of antibody combining site capable of immunoreacting with a particular antigen.

25

**Multimeric:** A polypeptide molecule comprising more than one polypeptide. A multimer may be dimeric and contain two polypeptides and a multimer may be trimeric and contain three polypeptides. Multimers may be homomeric and contain two or more identical polypeptides or a multimer may be heteromeric and contain two or more nonidentical polypeptides.

30

**Polyclonal antibody:** Polyclonal antibodies is a mixture of antibody molecules recognising a specific given antigen, hence polyclonal antibodies may recognise different epitopes within said antigen.

35

Polypeptide: The phrase polypeptide refers to a molecule comprising amino acid residues which do not contain linkages other than amide linkages between adjacent amino acid residues.

5

Receptor: A receptor is a molecule, such as a protein, glycoprotein and the like, that can specifically (non-randomly) bind to another molecule.

10

Secretagogue: A substance or chemical moiety capable of binding and activating the GHS 1a receptor.

15

Specificity: The term specificity refers to the number of potential antigen binding sites which immunoreact with (specifically bind to) a given polypeptide. The polypeptide may be a single polypeptide or may be two or more polypeptides joined by disulfide bonding.

20

Surfactant molecule: Molecule comprising a hydrophobic part and a hydrophilic part, i.e. molecule capable of being present in the interphase between a lipophilic phase and a hydrophilic phase.

### **Secretagogues**

25

A growth hormone secretagogue according to the invention is used in its normal meaning, i.e. a substance capable of stimulating growth hormone release. In the present context, a secretagogue is defined by its ability of binding GHS-R 1a, and more preferably activating the receptor.

30

A secretagogue according to the invention is preferably selected from the group of:  
L-692-429, L-692-585 (Benzoelactam compounds)  
MK677 (Spiroindaner)  
G-7203, G-7039, G-7502 (Isonipectic acid peptidomimetic)  
NN703, ipamorelin

35

In particular the secretagogue is a ghrelin-like compound, including 28 aa human ghrelin.

### **Subcutaneous administration**

5 It is important to note that ghrelin receptors are found in a number of places in the organism besides on the GH producing cells and in the hypothalamic centers for appetite etc. control. In the CNS these receptors are tuned to receiving signals from local ghrelin containing neurons. Peripherally secreted or artificially administered ghrelin might not reach such sites due to the blood brain barrier, however currently available so-called GH secretagogues, which are small organic compounds such as  
10 MK-0677, will pass the blood brain barrier and also reach these sites – and consequently have the danger of causing unwanted side effects. Thus such compounds which do have the advantage of being for example orally active will not be optimal for mimicking the natural pre-meal, appetite inducing surge of ghrelin, since they will reach basically all ghrelin receptors in the body. In contrast, by using the natural  
15 peptide, ghrelin itself or homologues thereof, and administering it peripherally – as in the present invention – it is ensured that only the relevant, appetite regulating ghrelin receptors are reached and stimulated.

20 Any parenteral administration form that will ensure that the ghrelin receptors which normally are the target for peripherally produced ghrelin in the premeal situation will be exposed to sufficient levels of the bioactive form of ghrelin to ensure robust and appropriate appetite stimulation without causing desensitization of the system may be part of the present invention. However, taken into consideration that the individuals to be treated possibly will have to receive treatment for a longer period, such as  
25 weeks or months, it is preferred that the administration form is well suited herefor.

Accordingly, it is preferred that the ghrelin-like compound according to the invention is administered subcutaneously in an amount sufficient to allow sufficient levels of the bioactive form of ghrelin, i.e. the acylated form, to reach the receptors in time,  
30 such as prior to the forthcoming meal.

The present invention preferably deals with methods for administering ghrelin in a way which mimics the physiologically pre-meal situation as closely as possible yet providing patients in need of increased food intake, for example fragile elderly, post  
35 operative patients, patients with lost appetite as part of cachexia for example pre-

precipitated by cancer, cardiac disease etc. with a sufficient extra stimulatory input to their appetite regulating ghrelin receptors, which normally are reached by ghrelin in the pre-meal situation.

## 5      **Bolus administration**

Furthermore, from a molecular pharmacological point-of-view it is important to note that it has been found that the ghrelin receptor normally is exposed to short-lived surges in the concentrations of the natural agonist ligand, ghrelin. The GHS-R 1a  
10      receptor belongs to the class of receptors, so-called G protein coupled receptors or 7TM receptors, which upon continued exposure to an agonist will be desensitized, internalized and down-regulated. These mechanisms, which are inherent to the overall signal transduction system, involve processes such as receptor phosphorylation (which in itself decreases the affinity of the receptor for the agonist) binding of  
15      inhibitory proteins such as arrestin (which sterically block the binding of signal transduction molecules such as G proteins). Other part of the agonist mediated desensitization process is receptor internalization (i.e. physical removal of the receptor from the cell surface where it could bind the agonist) as well as receptor down regulation (i.e. decreased production / expression of the receptor). Receptor internalization  
20      could after short-lived exposure of the receptor to agonist be followed by a re-sensitization process, where the receptor is dephosphorylated and recycled to the cell surface to be used again. Without being bound by theory, it is believed that, upon prolonged stimulation, which would occur for example during a long-lasting continuous infusion of the agonist, the receptor down-regulation process ensures  
25      that the target cell is adjusted in its signal transduction system etc. to this situation.

The present invention provides a procedure for an optimal administration of ghrelin to patients in order to obtain a maximal response and avoid for example desensitization mechanisms.

30

Accordingly, the present invention relates in one aspect to administration of the ghrelin-like compound in boluses, preferably a bolus prior to each main meal. It has been found, in contrary to the prolonged administration processes in the prior art, that a bolus administration leads to not only stimulation of appetite, but also to  
35      stimulation of feed intake and more important to stimulation of weight gain.

Without being bound by theory, it is believed that premeal subcutaneous injection, intravenous injection or short-term infusions of appropriate doses of ghrelin will ensure that a robust stimulation of appetite inducing ghrelin receptors will be obtained with minimal constraint to the mobility etc. of the patient. Thus for example patients with hip fractures can in the post operative situation be treated in the premeal period and if required during the meal as such, but will be free to move around and participate in the important post operative physiotherapeutic regimens.

## 10 Ghrelin-like compound

A ghrelin-like compound according to the invention described herein is a compound comprising a structure defined by formula I

15  $Z^1 - (X^1)_m - (X^2) - (X^3)_n - Z^2$ , wherein

$Z^1$  is an optionally present protecting group

each  $X^1$  is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

$X^2$  is any amino acid selected from naturally occurring and synthetic occurring amino acids, said amino acid being modified with a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

25 each  $X^3$  is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

wherein one or more of  $X^1$  and  $X^3$  optionally may be modified by a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

$Z^2$  is an optionally present protecting group,

m is an integer in the range of from 1-10

35

n is 0 or an integer in the range of from 1-35.

Accordingly, the term "ghrelin-like compound" includes the naturally occurring 28 aa human ghrelin, the amino acid of which is shown in SEQ ID NO: 1, as well as the naturally occurring 27 aa human ghrelin, the amino acid of which is shown in SEQ ID NO: 2. Thus, the present invention relates to the use of ghrelin or a peptide homologous thereto. Ghrelin is described by Kojima in Nature (1999), vol. 402,656-660.

The present invention includes diastereomers as well as their racemic and resolved enantiomerically pure forms. Ghrelin-like compounds can contain D-amino acids, L-amino acids, alpha-amino acid, beta-amino acid, gamma-amino acid, natural amino acid and synthetic amino acid or the like or a combination thereof. Preferably, amino acids present in a ghrelin-like compound are the L-enantiomer.

The ghrelin-like compound comprises an amino acid modified with a bulky hydrophobic group. The number of amino acids N-terminally to the modified amino acid is preferably within the range of from 1-9. Accordingly, m is preferably an integer in the range of from 1-9, such as of from 1-8, such as of from 1-7, such as of from 1-6, such as of from 1-5, such as of from 1-4, such as of from 1-3, such as of from 1-2, such as 2.

It is more preferred that the number of amino acids N-terminally to the modified amino acid is low, such as of from 1-3, such as of from 1-2. Most preferably 2 amino acids are positioned N-terminal to the modified amino acid.

In a preferred embodiment  $(X^1)_m$  has a Gly residue in the N-terminal part of the sequence. Accordingly, in preferred embodiment  $(X^1)_m$  is selected from the sequences:

Gly, Gly-Ser, Gly-Cys, Gly-Lys, Gly-Asp, Gly-Glu, Gly-Arg, Gly-His, Gly-Asn, Gly-Gln, Gly-Thr, and Gly-Tyr.

More preferably  $(X^1)_m$  is selected from , Gly-Ser, and Gly-Cys, most preferably from Gly-Ser.

In another words, in a preferred embodiment the ghrelin-like compound is selected from a compound of

formula II  $Z^1 - \text{Gly} - (X^1)_{m-1} - (X^2) - (X^3)_n - Z^2$ ,

formula III  $Z^1 - \text{Gly} - \text{Ser} - (X^2) - (X^3)_n - Z^2$ , and

formula IV  $Z^1 - \text{Gly} - (X^2) - (X^3)_n - Z^2$ .

And more preferably the ghrelin-like compound has formula III.

As described above,  $X^2$  may be any amino acid modified with a bulky hydrophobic group. In particular  $X^2$  is selected from the group of modified Ser, Cys, Asp, Lys, Trp, Phe, Ile, and Leu. More preferably  $X^2$  is selected from the group of modified Ser, modified Cys and modified Lys, and most preferably  $X^2$  is modified Ser.

Furthermore,  $(X^1)_m - (X^2)$  is preferably Gly-Xaa-Ser\*, or Gly-Xaa-Cys\*, wherein Xaa is any amino acid, more preferably  $(X^1)_m - (X^2)$  is Gly-Ser-Ser\*, or Gly-Ser-Cys\*, wherein \* indicates that the amino acid residue is modified with a bulky hydrophobic group.

$(X^3)_n$  preferably comprises a sequences which is a fragment of ghrelin, or a variant of a fragment of ghrelin, such as human ghrelin. Accordingly,  $(X^3)_n$  preferably comprises a sequence selected from one or more of the sequences shown below:

Phe Leu Ser Pro Glu His Gln

Phe Leu Ser Pro Glu His

Phe Leu Ser Pro Glu

Phe Leu Ser Pro

Phe Leu Ser

Phe Leu

Phe

In a preferred embodiment the length of the ghrelin-like compound is substantially similar to the length of human ghrelin, i.e. 27 or 28 amino acids. Accordingly, n is

preferably an integer in the range of from 1-25, such as of from 1-24, such as from 1-15, such as of from 1-10, or such as of from 10-25, such as of from 10-24, such as of from 15-25, such as of from 15-24.

- 5  $(X^3)_n$  may be selected from any fragment of ghrelin, such as human ghrelin, and accordingly,  $(X^3)_n$  may be selected from one or more of the sequences shown below or a homologue thereof:

10 Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Ala Lys Leu Gln Pro Arg  
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Ala Lys Leu Gln Pro  
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Ala Lys Leu Gln  
15 Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Ala Lys Leu  
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Ala Lys  
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Ala  
20 Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro  
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys  
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys  
25 Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser  
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu  
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys  
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg  
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln  
30 Phe Leu Ser Pro Glu His Gln Arg Val Gln  
Phe Leu Ser Pro Glu His Gln Arg Val  
Phe Leu Ser Pro Glu His Gln Arg  
Phe Leu Ser Pro Glu His Gln  
Phe Leu Ser Pro Glu His  
35 Phe Leu Ser Pro Glu



Phe Leu Ser Pro

Phe Leu Ser

Phe Leu

Phe

5

Or selected from

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Ala Lys Leu Gln Pro Arg

10

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Ala Lys Leu Gln Pro

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Ala Lys Leu Gln

15

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Ala Lys Leu

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Ala Lys

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Ala

20

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser

25

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln

Phe Leu Ser Pro Glu His Gln Lys Val Gln

30

Phe Leu Ser Pro Glu His Gln Lys Val

Phe Leu Ser Pro Glu His Gln Lys

Or selected from

Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
 Ala Lys Leu Gln Pro Arg  
 Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
 Ala Lys Leu Gln Pro  
 5 Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
 Ala Lys Leu Gln  
 Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
 Ala Lys Leu  
 Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
 10 Ala Lys  
 Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
 Ala  
 Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
 Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro  
 15 Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys  
 Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys  
 Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser  
 Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu  
 Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys  
 20 Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg  
 Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln  
 Phe Leu Ser Pro Glu His Gln Arg Ala Gln  
 Phe Leu Ser Pro Glu His Gln Arg Ala

25 Or selected from

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
 Ala Lys Leu Gln Pro Arg  
 Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
 30 Ala Lys Leu Gln Pro  
 Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
 Ala Lys Leu Gln  
 Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
 Ala Lys Leu

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
 Ala Lys  
 Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
 Ala  
 5 Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
 Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro  
 Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys  
 Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys  
 Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser  
 10 Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu  
 Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys  
 Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg  
 Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln  
 Phe Leu Ser Pro Glu His Gln Lys Ala Gln  
 15 Phe Leu Ser Pro Glu His Gln Lys Ala

In another embodiment  $(X^3)_n$  preferably comprises or consists of a sequence selected from the sequences

20 Phe Leu Ser Pro Glu His Gln  
 Phe Leu Ser Pro Glu His  
 Phe Leu Ser Pro Glu  
 Phe Leu Ser Pro  
 Phe Leu Ser  
 25 Phe Leu  
 Phe

### Functionality

30 The ghrelin-like compound described herein are active at the GHS receptor. The compounds can bind to the receptor, and preferably, stimulate receptor activity.

GHS receptor activity can be measured using different techniques such as detecting  
 a change in the intracellular conformation of the GHS receptor, in the G-protein cou-  
 35 pled activities, and/or in the intracellular messengers.

One simple measure of the ability of a ghrelin like compound to activate the ghrelin receptor is to measure its EC<sub>50</sub>, i.e. the dose at which the compound is able to activates the signalling of the receptor to half of the maximal effect of the compound.

5 The ghrelin receptor can either be expressed endogenously on primary cells cultures, for example pituitary cells, or heterologously expressed on cells transfected with the ghrelin receptor. Whole cell assays or assays using membranes prepared from either of these cell types can be used depending on the type of assay.

10 As the ghrelin receptor is generally believed to be primarily coupled to the Gq signalling pathway, any suitable assay which monitor activity in the Gq/G11 signalling pathway can be used, for example:

15 1) an assay measuring the activation of Gq / G11 performed for example by measurement of GTPγS binding combined with, e.g., anti-Gα-q or -11 antibody precipitation in order to increase the signal to noise ratio. This assay may also detect coupling to other G-proteins than Gq/11.

20 2) An assay which measure the activity of phospholipase C (PLC) one of the first down-stream effector molecules in the pathway, for example by measuring the accumulation of inositol phosphate which is one of the products of PLC.

25 3) More down stream in the signalling cascade is the mobilization of calcium from the intracellular stores

4) Further more down stream signalling molecules such as the activity of different kinds of MAP kinases (p38, jun, ect.), NF-κ-B translocation and CRE driven gene transcription may also be measured.

30 Alternatively binding of fluorescently tagged arrestin to the activated ghrelin receptor may also be used.

In one embodiment the binding of a compound to the receptor GHS-R 1A can be measured by the use of the assay described herein above.

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A ghrelin-like compound according to the invention preferably has at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, functional activity relative to 28 aa human ghrelin as determined using the assay described herein above, and/or an EC50 greater than about 1,000, greater than about 100, or greater than about 50, or greater than about 10. Greater refers to potency and thus indicates a lesser amount is needed to achieve binding inhibition.

In one embodiment of the invention the compound has a potency (EC50) on the GHS-R 1A of less than 500 nM. In another embodiment the compound has a potency (EC50) on the GHS-R 1A of less than 100 nM, such as less than 80 nM, for example less than 60 nM, such as less than 40 nM, for example less than 20 nM, such as less than 10 nM, for example less than 5 nM, such as less than 1 nM, for example less than 0.5 nM, such as less than 0.1 nM, for example less than 0.05 nM, such as less than 0.01 nM.

In a further embodiment the dissociation constant (Kd) of the compound is less than 500 nM. In a still further embodiment the dissociation constant (Kd) of the ligand is less than 100 nM, such as less than 80 nM, for example less than 60 nM, such as less than 40 nM, for example less than 20 nM, such as less than 10 nM, for example less than 5 nM, such as less than 1 nM, for example less than 0.5 nM, such as less than 0.1 nM, for example less than 0.05 nM, such as less than 0.01 nM.

Binding assays can be performed using recombinantly produced GHS receptor polypeptides present in different environments. Such environments include, for example, cell extracts and purified cell extracts containing the GHS receptor polypeptide expressed from recombinant nucleic acid or naturally occurring nucleic acid; and also include, for example, the use of a purified GHS receptor polypeptide produced by recombinant means or from naturally occurring nucleic acid which is introduced into a different environment.

Using a recombinantly expressed GHS receptor offers several advantages such as the ability to express the receptor in a defined cell system so that a response to a compound at the GHS receptor can more readily be differentiated from responses at other receptors. For example, the GHS receptor can be expressed in a cell line such as HEK 293, COS 7, and CHO not normally expressing the receptor by an

expression vector, wherein the same cell line without the expression vector can act as a control.

### **Identity and homology**

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The term "identity" or "homology" shall be construed to mean the percentage of amino acid residues in the candidate sequence that are identical with the residue of a corresponding sequence to which it is compared, after aligning the sequences and introducing gaps, if necessary to achieve the maximum percent identity for the entire sequence, and not considering any conservative substitutions as part of the sequence identity. Neither N- or C-terminal extensions nor insertions shall be construed as reducing identity or homology.. Methods and computer programs for the alignment are well known in the art. Sequence identity may be measured using sequence analysis software (e.g., Sequence Analysis Software Package, Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Ave., Madison, Wis. 53705). This software matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications.

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A homologue of one or more of the sequences specified herein may vary in one or more amino acids as compared to the sequences defined, but is capable of performing the same function, i.e. a homologue may be envisaged as a functional equivalent of a predetermined sequence.

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As described above a homologue of any of the predetermined sequences herein may be defined as:

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- i) homologues comprising an amino acid sequence capable of being recognised by an antibody, said antibody also recognising the 28 aa human ghrelin, preferably the acylated 28 aa human ghrelin, and/or
- ii) homologues comprising an amino acid sequence capable of binding selectively to GHS-R 1a, and/or

- iii) homologues having a substantially similar or higher binding affinity to GHS-R 1a than the 28 aa human ghrelin, preferably the acylated 28 aa human ghrelin.

5 In the above examples, the 28 aa human ghrelin has the sequence shown in SEQ ID NO: 1, and when acylated is acylated in position 3.

10 The antibodies used herein may be antibodies binding the N-terminal part of ghrelin or the C-terminal part of ghrelin, preferably the N-terminal part of ghrelin. The antibodies may be antibodies as described in Ariyasu et al. "Delayed short-term secretory regulation of ghrelin in obese animals: Evidenced by a specific RIA for the active form of ghrelin, Endocrinology 143(9):3341-3350, 2002.

15 Examples of homologues comprises one or more conservative amino acid substitutions including one or more conservative amino acid substitutions within the same group of predetermined amino acids, or a plurality of conservative amino acid substitutions, wherein each conservative substitution is generated by substitution within a different group of predetermined amino acids.

20 Homologues may thus comprise conservative substitutions independently of one another, wherein at least one glycine (Gly) of said homologue is substituted with an amino acid selected from the group of amino acids consisting of Ala, Val, Leu, and Ile, and independently thereof, homologues, wherein at least one of said alanines (Ala) of said homologue thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Val, Leu, and Ile, and independently thereof, homologues, wherein at least one valine (Val) of said homologue thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Leu, and Ile, and independently thereof, homologues thereof, wherein at least one of said leucines (Leu) of said homologue thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Val, and Ile, and independently thereof, homologues thereof, wherein at least one isoleucine (Ile) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Val and Leu, and independently thereof, homologues thereof wherein at least one of said aspartic acids (Asp) of said homologue thereof is substituted with an amino acid selected from the group of

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amino acids consisting of Glu, Asn, and Gln, and independently thereof, homologues thereof, wherein at least one of said phenylalanines (Phe) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Tyr, Trp, His, Pro, and preferably selected from the group of amino acids consisting of Tyr and Trp, and independently thereof, homologues thereof, wherein at least one of said tyrosines (Tyr) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Phe, Trp, His, Pro, preferably an amino acid selected from the group of amino acids consisting of Phe and Trp, and independently thereof, homologues thereof, wherein at least one of said arginines (Arg) of said fragment is substituted with an amino acid selected from the group of amino acids consisting of Lys and His, and independently thereof, homologues thereof, wherein at least one lysine (Lys) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Arg and His, and independently thereof, homologues thereof, wherein at least one of said asparagines (Asn) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Gln, and independently thereof, homologues thereof, wherein at least one glutamine (Gln) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Asn, and independently thereof, homologues thereof, wherein at least one proline (Pro) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Phe, Tyr, Trp, and His, and independently thereof, homologues thereof, wherein at least one of said cysteines (Cys) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, Lys, Arg, His, Asn, Gln, Ser, Thr, and Tyr.

Conservative substitutions may be introduced in any position of a preferred predetermined sequence. It may however also be desirable to introduce non-conservative substitutions, particularly, but not limited to, a non-conservative substitution in any one or more positions.

A non-conservative substitution leading to the formation of a functionally equivalent homologue of the sequences herein would for example 1) differ substantially in polarity, for example a residue with a non-polar side chain (Ala, Leu, Pro, Trp, Val, Ile, Leu, Phe or Met) substituted for a residue with a polar side chain such as Gly, Ser,



Thr, Cys, Tyr, Asn, or Gln or a charged amino acid such as Asp, Glu, Arg, or Lys, or substituting a charged or a polar residue for a non-polar one; and/or ii) differ substantially in its effect on polypeptide backbone orientation such as substitution of or for Pro or Gly by another residue; and/or iii) differ substantially in electric charge, for example substitution of a negatively charged residue such as Glu or Asp for a positively charged residue such as Lys, His or Arg (and vice versa); and/or iv) differ substantially in steric bulk, for example substitution of a bulky residue such as His, Trp, Phe or Tyr for one having a minor side chain, e.g. Ala, Gly or Ser (and vice versa).

Substitution of amino acids may in one embodiment be made based upon their hydrophobicity and hydrophilicity values and the relative similarity of the amino acid side-chain substituents, including charge, size, and the like. Exemplary amino acid substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

In a preferred embodiment the binding domain comprises a homologue having an amino acid sequence at least 60 % homologous to SEQ ID NO 1.

More preferably the homology is at least 65 %, such as at least 70 % homologous, such as at least 75 % homologous, such as at least 80 % homologous, such as at least 85 % homologous, such as at least 90 % homologous, such as at least 95 % homologous, such as at least 98 % homologous to SEQ ID NO 1.

In a more preferred embodiment the percentages mentioned above relates to the identity of the sequence of a homologue as compared to SEQ ID NO 1.

Homologues to SEQ ID NO: 1 may be 27 aa human ghrelin SEQ ID NO: 2, rat ghrelin SEQ ID NO: 3. Other homologues are the variants described in EP 1197496 (Kangawa) incorporated herein by reference.

#### **Bulky hydrophobic group**

The bulky hydrophobic group of the ghrelin-like compound according to the invention is any bulky hydrophobic group capable of providing the des-acylated 28 aa human ghrelin with binding affinity to GHS-R 1a when the Ser residue in position 3 is modified with the bulky hydrophobic group.

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When the amino acid being modified contains e.g. - OH, -SH, -NH or -NH<sub>2</sub> as a substituent group in a side chain thereof, a group formed by acylating such a substituent group is preferred. The mode of linkage may thus be selected from the group consisting of ester, ether, thioester, thioether, amide and carbamide.

10

For example, if the modified amino acid is serine, threonine, tyrosine or oxyproline, the amino acid has a hydroxyl group in the side chain. If the modified amino acid is cysteine, the amino acid has a mercapto group in the side chain. If the modified amino acid is lysine, arginine, histidine, tryptophan, proline or oxyproline, it has an amino group or imino group in the side chain.

15

The hydroxyl group, mercapto group, amino group and imino group described above may thus have been chemically modified. That is, the hydroxyl group or mercapto group may be etherized, esterified, thioetherified or thioesterified. The imino group may have been iminoetherified, iminothioetherified or alkylated. The amino group may have been amidated, thioamidated or carbamidated.

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Further, the mercapto group may have been disulfidated, the imino group may have been amidated or thioamidated, and the amino group may have been alkylated or thiocarbamidated.

25

In a preferred embodiment the modified amino acid is Ser coupled through an ester linkage to the hydrophobic group.

30

The hydrophobic group may be any group with a saturated or unsaturated alkyl or acyl group containing one or more carbon atoms. In one embodiment the bulky hydrophobic group is an acyl group, including groups formed by removing a hydroxyl group from an organic carboxylic acid, organic sulfonic acid or organic phosphoric acid. The organic carboxylic acid includes e.g. fatty acids, and the number of carbon

atoms thereof is preferably 1 to 35. In the organic sulfonic acid or organic phosphoric acid, the number of carbon atoms thereof is preferably 1 to 35.

5 Accordingly, the acyl group is preferably selected from a C1-C35 acyl group, such as a C1 – C20 acyl group, such as a C1 – C15 acyl group, such as a C6 – C15 acyl group, such as a C6 – C12 acyl group, such as a C8 – C12 acyl group.

10 More preferably the acyl group is selected from the group of C7 acyl group, C8 acyl group, C9 acyl group, C10 acyl group, C11 acyl group, and C12 acyl group. Such acyl group may be formed from octanoic acid (preferably caprylic acid), decanoic acid (preferably capric acid), or dodecanoic acid (preferably lauric acid), as well as monoene or polyene fatty acids thereof.

15 In one embodiment the acyl group is selected from the group of C8 acyl group, and C10 acyl group. Such acyl groups may be formed from octanoic acid (preferably caprylic acid), or decanoic acid (preferably capric acid).

20 In another embodiment the acyl group is selected from the group of C7 acyl group, C9 acyl group, and C11 acyl group, such as from the group of C9 acyl group and C11 acyl group.

25 Furthermore, the modified amino acid may be any amino acid wherein a group is modified as described in EP 1 197 496 (Kangawa), which is hereby incorporated by reference.

### **Protecting group**

30 The ghrelin-like compound according to the invention may comprise a protecting group at the N-terminus or the C-terminus or at both.

A protecting group covalently joined to the N-terminal amino group reduces the reactivity of the amino terminus under in vivo conditions. Amino protecting groups include - C1-10 alkyl, -C1-10 substituted alkyl, -C2-10 alkenyl, -C2-10 substituted alkenyl, aryl, -C1-6 alkyl aryl, -C(O)- (CH<sub>2</sub>)<sup>1-6</sup>-COOH, -C(O)-C1-6 alkyl, -C(O)-aryl,

-C (O)-O-C1-6 alkyl, or-C (O)-O-aryl. Preferably, the amino terminus protecting group is acetyl, propyl, succinyl, benzyl, benzyloxycarbonyl or tbutyloxycarbonyl.

5 A protecting group covalently joined to the C-terminal carboxy group reduces the reactivity of the carboxy terminus under in vivo conditions. The carboxy terminus protecting group is preferably attached to the  $\alpha$ -carbonyl group of the last amino acid. Carboxy terminus protecting groups include amide, methylamide, and ethylamide.

## 10 **Conjugates**

The ghrelin-like compound may also be administered in a form, wherein the ghrelin-like compound is conjugated to another entity.

15 For example the compound may be a conjugate of ghrelin or a derivative or homologue thereof and another peptide, such as a peptide having effect on nociceptin receptor ORL1. In one embodiment the conjugate is a conjugate of ghrelin or a derivative or homologue thereof and Ac-RYY(RK)(WI)RK)-NH<sub>2</sub>, where the brackets show allowable variation of amino acid residues. Examples peptides in the conjugate may also be found in US patent application 2003040472

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## **Method for production**

Ghrelin-like compounds can be produced using techniques well known in the art. For example, a polypeptide region of a ghrelin-like compound can be chemically or biochemically synthesized and modified. Techniques for chemical synthesis of polypeptides are well known in the art. (See e. g., Vincent in Peptide and Protein Drug Delivery, New York, N. Y., Dekker, 1990.) Examples of techniques for biochemical synthesis involving the introduction of a nucleic acid into a cell and expression of nucleic acids are provided in Ausubel, Current Protocols in Molecular Biology, John Wiley, 1987-1998, and Sambrook et al., in Molecular Cloning, A Laboratory Manual, 2 d Edition, Cold Spring Harbor Laboratory Press, 1989.

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## **Pharmaceutical composition**

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In one aspect the invention relates to a pharmaceutical composition comprising a ghrelin-like compound as defined herein, wherein the bulky hydrophobic group is an acyl group selected from the group of C7 acyl group, C9 acyl group, and C11 acyl group, such as from the group of C9 acyl group and C11 acyl group.

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In another embodiment the invention relates to a pharmaceutical composition comprising a mixture of at least two different ghrelin-like compounds, such as a mixture of a ghrelin-like compound being acylated with a C8 acyl and a ghrelin-like compound being acylated with a C10 acyl. Without being bound by theory it is believed that such a mixture will have a longer half-life in plasma, or will more closely resemble the natural situation.

10

In yet another embodiment, the pharmaceutical composition comprises acylated ghrelin-like compounds, optionally compounds having different acyl chain lengths preferably selected from the group of C7 acyl group, C9 acyl group, and C11 acyl group, such as from the group of C9 acyl group and C11 acyl group, further optionally in combination with a desacylated Ghrelin-like compound.

15

In another aspect the invention relates to a pharmaceutical composition comprising any compound as defined above or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable carriers, vehicles and/or excipients said composition further comprising transport molecules. The transport molecules are primarily added in order to increase the half-life of the acylated compound, preventing premature des-acylation, since the des-acylated ghrelin is not active at the GHS-R 1a.

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Transport molecules act by having incorporated into or anchored to it the compound according to the invention.

25

Any suitable transport molecules known to the skilled person may be used. Examples of transport molecules may be liposomes, micelles, and/or microspheres.

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A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al., Ann. Rev. Biophys. Bioeng. 9:467 (1980), U.S. Pat. Nos. 4, 235,871, 4,501,728 and 4,837,028, all of which are incorporated herein by reference. One method produces multilamellar vesicles of heterogeneous sizes. In this method, the

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- vesicle-forming lipids are dissolved in a suitable organic solvent or solvent system and dried under vacuum or an inert gas to form a thin lipid film. If desired, the film may be redissolved in a suitable solvent, such as tertiary butanol, and then lyophilized to form a more homogeneous lipid mixture which is in a more easily hydrated powderlike form. This film is covered with an aqueous solution of the targeted drug and the targeting component and allowed to hydrate, typically over a 15-60 minute period with agitation. The size distribution of the resulting multilamellar vesicles can be shifted toward smaller sizes by hydrating the lipids under more vigorous agitation conditions or by adding solubilizing detergents such as deoxycholate. Additionally, the liposome suspension may include lipid-protective agents which protect lipids against free-radical and lipid-peroxidative damages on storage. Lipophilic free-radical quenchers, such as .alpha. tocopherol and water-soluble iron-specific chelators, such as ferrioxianine, are preferred.
- Micelles are formed by surfactants (molecules that contain a hydrophobic portion and one or more ionic or otherwise strongly hydrophilic groups) in aqueous solution. As the concentration of a solid surfactant increases, its monolayers adsorbed at the air/water or glass/water interfaces become so tightly packed that further occupancy requires excessive compression of the surfactant molecules already in the two monolayers. Further increments in the amount of dissolved surfactant beyond that concentration cause amounts equivalent to the new molecules to aggregate into micelles. This process begins at a characteristic concentration called "critical micelle concentration".
- The shape of micelles formed in dilute surfactant solutions is approximately spherical. The polar head groups of the surfactant molecules are arranged in an outer spherical shell whereas their hydrocarbon chains are oriented toward the center, forming a spherical core for the micelle. The hydrocarbon chains are randomly coiled and entangled and the micellar interior has a nonpolar, liquid-like character. In the micelles of polyoxyethylated nonionic detergents, the polyoxyethylene moieties are oriented outward and permeated by water. This arrangement is energetically favorable since the hydrophilic head groups are in contact with water and the hydrocarbon moieties are removed from the aqueous medium and partly shielded from contact with water by the polar head groups. The

hydrocarbon tails of the surfactant molecules, located in the interior of the micelle, interact with one another by weak van der Waals forces.

5 The size of a micelle or its aggregation number is governed largely by geometric factors. The radius of the hydrocarbon core cannot exceed the length of the extended hydrocarbon chain of the surfactant molecule. Therefore, increasing the chain length or ascending homologous series increases the aggregation number of spherical micelles. If the surfactant concentration is increased beyond a few percent and if electrolytes are added (in the case of ionic surfactants) or the temperature is  
10 raised (in the case of nonionic surfactants), the micelles increase in size. Under these conditions, the micelles are too large to remain spherical and become ellipsoidal, cylindrical or finally lamellar in shape.

15 Common surfactants well known to one of skill in the art can be used in the micelles of the present invention. Suitable surfactants include sodium laurate, sodium oleate, sodium lauryl sulfate, octaoxyethylene glycol monododecyl ether, octoxynol 9 and PLURONIC F-127 (Wyandotte Chemicals Corp.). Preferred surfactants are nonionic polyoxyethylene and polyoxypropylene detergents compatible with IV injection such as, TWEEN-80, PLURONIC F-68, n-octyl-.beta.-D-glucopyranoside,  
20 and the like. In addition, phospholipids, such as those described for use in the production of liposomes, may also be used for micelle formation.

### **Administration**

25 Suitable dosing regimens are preferably determined taking into account factors well known in the art including type of subject being dosed; age, weight, sex and medical condition of the subject; the route of administration; the renal and hepatic function of the subject; the desired effect; and the particular compound employed.

30 Optimal precision in achieving concentrations of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug.

As described above, in one aspect of the invention, the ghrelin-like compound is administered subcutaneously.

5 In another aspect the ghrelin-like compound is administered as a premeal bolus, wherein the administration form may be any suitable parenteral form.

In a preferred embodiment the ghrelin-like compound is administered subcutaneously in a premeal bolus.

10 The ghrelin-like compound can also be administered during a meal as a bolus. The mode of administration during a meal includes subcutaneous administration, such as a subcutaneously administered bolus.

15 Pharmaceutical compositions for parenteral administration include sterile aqueous and non-aqueous injectable solutions, dispersions, suspensions or emulsions, as well as sterile powders to be reconstituted in sterile injectable solutions or dispersions prior to use.

20 Other suitable administration forms include suppositories, sprays, ointments, cremes, gels, inhalants, dermal patches, implants, pills, tablets, lozenges and capsules.

25 A typical dosage of a compound employed according to the invention is in a concentration equivalent to from 10 ng to 10 mg ghrelin per kg bodyweight. The concentrations and amounts herein are given in equivalents of amount ghrelin, wherein the ghrelin is the 28 aa human ghrelin. Equivalents may be tested a method as described in Example 2.

30 In a preferred embodiment the medicament is administered in a concentration equivalent to from 0.1 µg to 1 mg ghrelin per kg bodyweight, such as from 0.5 µg to 0.5 mg ghrelin per kg bodyweight, such as from 1.0 µg to 0.1 mg ghrelin per kg bodyweight, such as from 1.0 µg to 50 µg ghrelin per kg bodyweight, such as from 1.0 µg to 10 µg ghrelin per kg bodyweight.



As described above, the ghrelin-like compound is preferably administered as a bolus. Accordingly, in one embodiment the medicament is administered as a bolus prior to a meal, said bolus comprising an amount of the ghrelin-like compound or a salt thereof equivalent to from 0.3  $\mu$ g to 600 mg ghrelin. More preferred the medicament is administered as a bolus prior to a meal, said bolus comprising an amount of the ghrelin-like compound or a salt thereof equivalent to from 2.0  $\mu$ g to 200 mg ghrelin, such as from 5.0  $\mu$ g to 100 mg ghrelin, such as from 10  $\mu$ g to 50 mg ghrelin, such as from 10  $\mu$ g to 5 mg ghrelin, such as from 10  $\mu$ g to 1.0 mg ghrelin.

It should be noted that the normal ghrelin response which occurs before a meal is a short-lived surge in plasma concentrations of ghrelin and that due to the relative short half life of the peptide an i.v. injection of ghrelin will ensure that a similar short-lived peak on ghrelin concentrations can be obtained. The administration route must ensure that the non-degraded, bioactive form of the peptide will be the dominating form in the circulation, which will reach the ghrelin receptors and stimulate these. Thus, in order to obtain the maximum effect of the medicament it is preferably administered from one to three times daily, each administration being within 45 minutes of a meal, such as within 30 minutes of a meal, such as within 25 minutes of a meal, such as within 20 minutes of a meal, such as within 15 minutes of a meal, such as within 10 minutes of a meal, such as within 5 minutes of a meal. More preferred the medicament is administered prior to each main meal, such as administered three times daily.

### Formulation

In a preferred aspect the present invention contemplates pharmaceutical compositions useful for practicing the therapeutic methods described herein. Pharmaceutical compositions of the present invention contain a physiologically tolerable carrier together with at least one species of ghrelin-like compound as described herein, dissolved or dispersed therein as an active ingredient. In a preferred embodiment, the pharmaceutical composition is not immunogenic when administered to a human individual for therapeutic purposes, unless that purpose is to induce an immune response.

In one aspect the invention relates to a pharmaceutical composition comprising at least one ghrelin-like compound as defined above. In a preferred embodiment the pharmaceutical composition comprises at least two different ghrelin-like compounds as defined above in order to increase the effect of the treatment. The difference may  
5 for example be compounds having different acylations as discussed above.

As used herein, the terms "pharmaceutically acceptable", "physiologically tolerable" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable  
10 of administration to or upon a human without the production of undesirable physiological effects such as nausea, dizziness, gastric upset and the like.

The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art. Typically such compositions are prepared as sterile injectables either as liquid solutions or suspensions,  
15 aqueous or non-aqueous, however, solid forms suitable for solution, or suspensions, in liquid prior to use can also be prepared. The preparation can also be emulsified.

The active ingredient can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like  
20 which enhance the effectiveness of the active ingredient.

The pharmaceutical composition of the present invention can include pharmaceutically acceptable salts of the compounds therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide).  
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Such salts include pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, ammonium salts and alkylated ammonium salts. Acid addition salts include salts of inorganic acids as well as organic acids. Representative  
35 examples of suitable inorganic acids include hydrochloric, hydrobromic, hydriodic,

phosphoric, sulphuric and nitric acids and the like. Representative examples of suitable organic acids include formic, acetic, trichloroacetic, trifluoroacetic, propionic, benzoic, cinnamic, citric, fumaric, glycolic, lactic, maleic, malic, malonic, mandelic, oxalic, picric, pyruvic, salicylic, succinic, methanesulfonic, ethanesulfonic, tartaric, ascorbic, pantoic, bismethylene salicylic, ethanedisulfonic, gluconic, citraconic, aspartic, stearic, palmitic, ethylenediaminetetraacetic (EDTA), p-aminobenzoic, glutamic, benzenesulfonic and p-toluenesulfonic acids and the like. Further examples of pharmaceutically acceptable inorganic or organic acid addition salts include the pharmaceutical acceptable salts listed in J. Pharm. Sci. 1977,66,2, which is incorporated herein by reference. Examples of metal salts include lithium, sodium, potassium and magnesium salts and the like.

Examples of ammonium and alkylated ammonium salts include ammonium, methylammonium, dimethylammonium, trimethylammonium, ethylammonium, hydroxyethylammonium, diethylammonium, butylammonium and tetramethylammonium salts and the like.

Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like.

Also included within the scope of compounds or pharmaceutical acceptable acid addition salts thereof in the context of the present invention are any hydrates (hydrated forms) thereof.

For parenteral administration, solutions of the present compounds in sterile aqueous solution, aqueous propylene glycol or sesame or peanut oil may be employed. Such aqueous solutions should be suitably buffered if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. The aqueous solutions are particularly suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. The sterile aqueous media employed are all readily available by standard techniques known to those skilled in the art.

Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, organic esters such as ethyl oleate, and water-oil emulsions.

5      Suitable pharmaceutical carriers include inert solid diluents or fillers, sterile aqueous solution and various organic solvents. Examples of solid carriers are lactose, terra alba, sucrose, cyclodextrin, talc, gelatine, agar, pectin, acacia, magnesium stearate, stearic acid or lower alkyl ethers of cellulose. Examples of liquid carriers are syrup,  
10      peanut oil, olive oil, phospholipids, fatty acids, fatty acid amines, polyoxyethylene or water. Administered by nasal aerosol or inhalation formulations may be prepared, for example, as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, employing fluorocarbons, and/or employing other solubilizing or dispersing agents.

15      The pharmaceutical compositions formed by combining the compounds of the invention and the pharmaceutical acceptable carriers are then readily administered in a variety of dosage forms suitable for the disclosed routes of administration. The formulations may conveniently be presented in unit dosage form by methods known in the art of pharmacy.

20      In a preferred embodiment of the invention the formulation comprises the ghrelin-like compound or a salt thereof as a lyophilisate and the formulation further comprises a solvent, said lyophilisate and said solvent being in separate compartments until administration.

25      In another embodiment the formulation is a solution of the ghrelin-like compound or a salt thereof.

30      In both embodiment the solvent may be any suitable solvents, such as described herein, and preferably the solvent is saline.

35      The invention also relates to a method for preparing a medicament or pharmaceutical composition comprising an compound of the invention, comprising admixing at least one ghrelin-like compound as defined above with a physiologically acceptable carrier.

In a still further aspect, the invention relates to a pharmaceutical composition comprising, as an active ingredient, a compound as defined above or a pharmaceutical acceptable salt thereof together with a pharmaceutical acceptable carrier.

5

Accordingly, the formulation may further include the transport molecules as described above.

10

In a further aspect of the invention the present compounds may be administered in combination with further pharmacologically active substances, e. g. an antidiabetic agent or other pharmacologically active material, including other compounds for the treatment and/or prevention of insulin resistance and diseases wherein insulin resistance is the pathophysiological mechanism. The combination may be in the form of kit-in-part systems, wherein the combined active substances may be used for simultaneous, sequential or separate administration.

15

As an example of a further pharmacologically active substance, the ghrelin-like compounds according to the invention can be administered in combination with a pharmaceutically effective amount of a growth hormone, including hGH.

20

Uses and combination treatments involving administration of the ghrelin-like compound according to the invention can also involve prophylaxis or treatment of lipodystrophy in combination with one or more of

25

a) prophylaxis and/or alleviation and/or treatment of a clinical depression, which combination treatment further comprises administering an antidepressant, a prodrug thereof, or a pharmaceutically acceptable salt of said antidepressant or said prodrug, and/or

30

b) prophylaxis and/or alleviation and/or treatment of an emetic condition, including nausea and vomiting, which combination treatment further comprises administering an antiemetic agent, a prodrug thereof, or a pharmaceutically acceptable salt of said antiemetic agent or said prodrug, and/or

c) prophylaxis and/or alleviation and/or treatment of a psychotic condition, which combination treatment further comprises administering an antipsychotic agent, a prodrug thereof or a pharmaceutically acceptable salt of said antipsychotic agent or said prodrug, and/or

5

d) prophylaxis and/or alleviation and/or treatment of anxiety, which combination treatment further comprises administering an antianxiety agent, a prodrug thereof or a pharmaceutically acceptable salt of said antianxiety agent or said prodrug, and/or

## 10 **Clinical Depression**

Clinical depression is characterized by a combination of symptoms that interfere with the ability to work, study, sleep, eat, and enjoy once pleasurable activities. Symptoms include: persistent sad or anxious mood; feelings of hopelessness or pessimism; feelings of guilt, worthlessness or helplessness; loss of interest in pleasure activities; decreased energy; difficulty concentrating, remembering, or making decisions; sleep abnormalities (e.g. insomnia); appetite and/or weight loss; thoughts of death or suicide; restlessness; and irritability.

15

Depression is a common disorder, occurring in approximately 10 percent of the U.S. population. Major depression is a leading cause of disability in the U.S. and worldwide, and a leading cause of days lost from work. Many causes of clinical depression having roots in the anatomy of the human brain. Neurotransmitter activity, genetic predisposition, and environmental factors are believed to be involved in the development of depression.

20

25

Diagnosis of depression is complicated, requiring a physical examination to rule out certain medications or medical conditions and a psychological examination to thoroughly evaluate the symptoms and determine how severely the symptoms have affected the life of the patient. Accordingly, depression is difficult to diagnose due to the variety of ways in which depression manifests itself.

30

In the above combination treatment, the antidepressant is preferably a norepinephrine reuptake inhibitor (NERI), a selective serotonin reuptake inhibitor (SSRI), a monoamine oxidase inhibitor (MAOI), a combined NERI/SSRI, or an atypical antide-

35

pressant, a prodrug of said antidepressant or a pharmaceutically acceptable salt of said antidepressant or said prodrug.

5 One preferred antidepressant is a selective serotonin reuptake inhibitor (SSRI), a prodrug thereof or a pharmaceutically acceptable salt of said SSRI or said prodrug. The SSRI is preferably citalopram, escitalopram, femoxetine, fluoxetine, fluvoxamine, indalpine, indeloxazine, milnacipran, paroxetine, sertraline, sibutramine or zimeldine, a prodrug of said SSRI or a pharmaceutically acceptable salt of said SSRI or said prodrug. Of the above, citalopram and escitalopram, a prodrug or a  
10 pharmaceutically acceptable salt thereof, are preferred in certain embodiments of combination treatments according to the present invention.

#### **Emesis, including nausea and vomiting**

15 Emesis can be caused by a number of factors, including mediation. Emesis can thus be an unavoidable side effect associated with many forms of medication. Preferred antiemetic agents according to the invention include meclizine hydrochloride, prochlorperazine, promethazine, trimethobenzamide hydrochloride and ondansetron hydrochloride.

20

#### **Clinical Psychotic Condition**

There are few psychiatric disorders in which clinical manifestations and symptoms can be correlated with a demonstrable pathology. The majority of mental illnesses  
25 are evaluated by observing changes in key behaviors such as thinking, mood, or social behavior. These alterations are difficult to ascertain and nearly impossible to quantify.

Preferred antipsychotic agents in accordance with the present invention are chlorpromazine, haloperidol, clozapine, loxapine, molindone hydrochloride, thiothixene,  
30 olanzapine, ziprasidone, ziprasidone hydrochloride, prochlorperazine, perphenazine, trifluoperazine hydrochloride and risperidone.

#### **Anxiety**

35

The combination methods of this invention are also directed to treatment, prevention, amelioration, alleviation, and of conditions of anxiety in a mammal, including a human being. The term anxiety covers generalized anxiety disorder, panic anxiety, obsessive compulsive disorder, social phobia, performance anxiety, post-traumatic stress disorder, acute stress reaction, adjustment disorders, hypochondriacal disorders, separation anxiety disorder, agoraphobia and specific phobias.

Preferred antianxiety agents are alprazolam, clonazepam, lorazepam, oxazepam, chlordiazepoxide hydrochloride, diazepam, buspirone hydrochloride, doxepin hydrochloride, hydroxyzine pamoate and clonazepam.

Further conditions of individual capable of being treatable in accordance with the present invention are bulimia nervosa, male erectile dysfunction, female sexual dysfunction, thyroid cancer, breast cancer, amelioration of ischemic nerve or muscle damage, as well as systemic lupus erythematosus.

The above medicaments are administered in pharmaceutically effective amounts, i.e. an administration involving a total amount of each active component of the medicament or pharmaceutical composition or method that is sufficient to show a meaningful patient benefit.

## Examples

### Example 1

#### Competition binding assays

Transfected COS-7 cells were transferred to culture plates one day after transfection at a density of  $1 \times 10^5$  cells per well aiming at 5 - 8 % binding of the radioactive ligand. Two days after transfection competition binding experiments were performed for 3 hours at 4EC using 25 pM of  $^{125}\text{I}$ -ghrelin (Amersham, Little Chalfont, UK). Binding assays were performed in 0.5 ml of a 50 mM Hepes buffer, pH 7.4, supplemented with 1 mM  $\text{CaCl}_2$ , 5 mM  $\text{MgCl}_2$ , and 0.1 % (w/v) bovine serum albumin, 40 :g/ml bacitracin. Non-specific binding was determined as the binding in the presence of 1 :M of unlabeled ghrelin. Cells were washed twice in 0.5 ml of ice-



cold buffer and 0.5-1 ml of lysis buffer (8 M Urea, 2 % NP40 in 3 M acetic acid) was added and the bound radioactivity was counted. Determinations were made in duplicate. Initial experiments showed that steady state binding was reached with the radioactive ligand under these conditions.

5

## **Example 2**

### **Receptor activation assays**

10 One simple measure of the ability of a ghrelin like compound to activate the ghrelin receptor is to measure its EC<sub>50</sub>, i.e. the dose at which the compound is able to activates the signalling of the receptor to half of the maximal effect of the compound. The ghrelin receptor can either be expressed endogenously on primary cells cul-  
15 tures, for example pituitary cells, or heterologously expressed on cells transfected with the ghrelin receptor. Whole cell assays or assays using membranes prepared form either of these cell types can be used depending on the type of assay.

As the ghrelin receptor is generally believed to be primarily coupled to the Gq signalling pathway, any suitable assay which monitor activity in the Gq/G11 signalling  
20 pathway can be used, for example:

- 1) an assay measuring the activation of Gq / G11 performed for example by measurement of GTPγS binding combined with, e.g., anti-Gα-q or -11 antibody precipitation in order to increase the signal to noise ratio. This assay may also de-  
25 tect coupling to other G-proteins than Gq/11.
- 2) An assay which measure the activity of phospholipase C (PLC) one of the first down-stream effector molecules in the pathway, for example by measuring the accumulation of inositol phosphate which is one of the products of PLC.  
30
- 3) More down stream in the signalling cascade is the mobilization of calcium from the intracellular stores

- 4) Further more down stream signalling molecules such as the activity of different kinds of MAP kinases (p38, jun, ect.), NF- $\kappa$ -B translocation and CRE driven gene transcription may also be measured.
- 5) Alternatively binding of fluorescently tagged arrestin to the activated ghrelin receptor may also be used.

### Example 3

#### Synthetic production of ghrelin-like compound

Amino acid derivatives and synthesis reagents, were obtained from commercial sources. Peptide chain extension was performed by mainly using Applied Biosystem 433A synthesizer produced by Perkin Elmer, and a protected peptide derivative-resin was constructed by the Boc or Fmoc method. The protected peptide resin obtained by the Boc method was deprotected with anhydrous hydrogen fluoride (HF) in the presence of p-cresol thereby releasing the peptide, which was then purified. The protected peptide resin obtained by the Fmoc method was deprotected with trifluoroacetic acid (TFA) or dilute TFA containing various scavengers, and the released peptide was purified. Purification was performed in reversed phase HPLC on a C4 or C18 column. The purity of the purified product was confirmed by reverse phase HPLC, and its structure was confirmed by amino acid composition analysis and mass spectrometry.

The peptide of the present invention is produced by a conventional peptide synthesis method. Specifically, synthesis of acylated or alkylated peptides is exemplified below. Further, human-derived ghrelin (which may be abbreviated hereinafter to hGhrelin) or rat-derived ghrelin (which may be abbreviated hereinafter to rGhrelin) was reacted with trypsin or chymotrypsin or both the enzymes successively to give the following ghrelin fragments: 19. Ghrelin (16-28), 20. hGhrelin (1-15), 21. rGhrelin (1-15), 23. hGhrelin (1-11), 24. rGhrelin (1-11), 25. Ghrelin (1-10), 26. Ghrelin (1-9), 27. Ghrelin (1-8), and 30. Ghrelin (1-4). Then, these fragments were isolated by analytical HPLC and measured for their activity. 41. [N-Acetyl]-Ghrelin (1-10) was prepared in a usual manner by treating Ghrelin (1-10) with N-acetylsuccinimide. Human and rat ghrelin may also be made by use of a natural material.

## Abbreviations

	HMP resin; 4-hydroxymethyl-phenoxyethyl resin
5	Fmoc amide resin; 4-(2', 4'-dimethoxyphenyl-Fmoc-aminomethyl) phenoxyacet- amido-ethyl resin
	PAM resin; phenylacetoamidomethyl resin
	HBTU; 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
	TBTU; 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
10	HOBt; 1-hydroxybenzotriazole
	DCC; dicyclohexylcarbodiimide
	DIPCI; diisopropylcarbodiimide
	TFA; trifluoroacetic acid
	DIPEA; diisopropylethylamine
15	TIPS; triisopropylsilane
	Fmoc; fluorenylmethoxycarbonyl
	Boc; t-butyloxycarbonyl
	Trt; trityl
	Bu; t-butyl
20	Pmc; 2,2,5,7,8-pentamethylchroman-6-sulfonyl
	Prl; propionyl
	PhPrl; phenylpropionyl
	Bzl; benzyl
	Bom; benzyloxymethyl
25	Tos; toluenesulfonyl
	Cl-Z; 2-chloro-benzyloxycarbonyl
	Pis; 2-phenylisopropyl
	Mtt; 4-methyltrityl
	DMF; N,N-dimethylformamide
30	NMP; N-methylpyrrolidone
	DMAP; 4-dimethylaminopyridine
	HOSu; N-hydroxysuccinimide
	Adod; 2-aminododecanoic acid
	Aib; 2-aminoisobutylic acid
35	Ape; 5-aminopentanoic acid

Cha; cyclohexylalanine

Dap; 2, 3-diaminopropionic acid

Nal; naphthylalanine

Nle; norleucine

5

Protecting amino acids used in synthesis

Fmoc method:

10 Boc-Gly, Fmoc-Gly, Fmoc-Ser (Bu), Fmoc-Ser (Trt), Fmoc-Glu (OBu), Fmoc-His  
(Boc), Fmoc-Gln (Trt), Fmoc-Arg (Pmc), Fmoc-Lys (Boc), Fmoc-Pro, Fmoc-Leu,  
Fmoc-Ala, Fmoc-Val, Fmoc-Phe, Fmoc-Phe, Fmoc-Ser (n-C<sub>8</sub>H<sub>17</sub>), Fmoc-Ser (n-  
C<sub>8</sub>H<sub>17</sub>), Fmoc-Cys (n-C<sub>8</sub>H<sub>17</sub>), Fmoc-Asp (OPis), Fmoc-Ser (Bzl), Fmoc-Cys (Trt),  
Fmoc-Dap (Octanoyl), Fmoc-2-Nal, Fmoc-2-Nal, Fmoc-Nle, Fmoc-Lys (Mtt), Fmoc-  
15 Aib-OH, Fmoc-Asp (O-C<sub>7</sub>H<sub>15</sub>)

Boc method:

Boc-Gly, Boc-Ser (Bzl), Boc-Ser (Ac), Boc-Ser (Pri), Boc-Glu (OBzl), Boc-His (Bom),  
Boc-Gln, Boc-Arg (Tos), Boc-Lys (Cl-Z), Boc-Pro, Boc-Leu, Boc-Ala, Boc-Val, Boc-  
20 Phe, Boc-Cys (n-C<sub>8</sub>H<sub>17</sub>), Boc-Ape Boc-Ser (n-C<sub>8</sub>H<sub>17</sub>)

Units used

(a) Analytical HPLC system

25

Unit: Shimadzu LC-10A System

Column: YMC PROTEIN-RP (4.6 mm phi x150 mm)

Column temperature: 40 DEG C

30 Eluent: A linear gradient of from 0 to 50 % acetonitrile for 20 minutes in 0.1% trifluo-  
roacetic acid

Flow rate: 1 mL/min.

Detection: UV (210 nm)

Injection volume: 10 to 100 µl

35

Preparative HPLC system

Unit: Waters 600 Multisolvent Delivery System

Columns:

- 5 YMC-Pack-ODS-A (5  $\mu$  m, 20 mmx250 mm)  
YMC-Pack-PROTEIN-RP (5  $\mu$  m, C4, 10 mmx250 mm)  
YMC-Pack PROTEIN-RP (5  $\mu$  m, C4, 20 mmx250 mm)  
YMC PROTEIN-RP (4.6 mm phi x150 mm)  
Eluent: A suitable linear gradient of acetonitrile concentration in 0.1 % trifluoroacetic acid
- 10 Flow rate: 10 mL/min. (for the column of an inner diameter of 20 mm), 3 mL/min. (for the column of an inner diameter of 10 mm), 1 mL/min. (for the column of an inner diameter of 4.6 mm) Detection: 210 nm, 260 nm  
Injection: 10 to 2000  $\mu$  l (2000  $\mu$  l or more was injected via a pump)

15

(c) Mass spectrometer

Unit: Finigan MAT TSQ700

Ion source: ESI

20

Detection ion mode: Positive

Spray voltage: 4.5 kV

Capillary temperature: 250 DEG C

Mobile phase: A mixture of 0.2% acetic acid and methanol (1 : 1)

Flow rate: 0.2 mL/min.

25

Scan range: m/z 300 to 1,500

(d) Analysis of amino acid sequence

Unit: Applied Biosystem 477A, 492 model sequencer manufactured by Perkin Elmer

30

(e) Analysis of amino acid composition

Unit: L-8500 model amino acid analyzer manufactured by Hitachi, Co., Ltd.

35

Sample: Unless otherwise specified, the sample was hydrolyzed with 6 M HCl at

110 DEG C for 24 hours in a sealed tube.

**Example of synthesis of a derivative having acyl serine (Fmoc method, carboxyl-terminal amide derivatives)**

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hGhrelin: GSS(CO-C7H15)FLSPEHQRVQQRKESKKPPAKLQPR

10 Fmoc-Arg(Pmc)-HMP-resin (403 mg, 0.25 mmol, ABI Co., Ltd) was treated with 20% piperazine for 20 minutes and subjected repeatedly to introduction of Fmoc-amino acid by HBTU/HOBt and elimination of Fmoc by piperazine sequentially to construct Fmoc-Ser(Bu)-Ser(Trt)-Phe-Leu-Ser(tBu)-Pro-Glu(OBu)-His(Boc)-Gln(Trt)-Arg(Pmc)-Val-Gln(Trt)-Gln(Trt)-Arg(Pmc)-Lys(Boc)-Glu(OBu)-Ser(Bu)-Lys(Boc)-Lys(Boc)-Pro-Pro-Ala-Lys(Boc)-Leu-Gln(Trt)-Pro-Arg(Pmc)-resin. After Boc-Gly was 15 finally introduced by DCC/HOBt, the resulting protected peptide resin (1.3 g) was treated with 1 % TFA-5 % TIPS-methylene chloride solution (15 mL) for 30 minutes. The peptide resin was filtrated, washed several times with methylene chloride (30 mL), and washed with 5 % DIEA (10 mL) and then with methylene chloride (30 mL). The resulting de-Trt peptide resin (about 1.3 g) was swollen with NMP (10 mL), and 20 octanoic acid (144.2 mg, 1.0 mmol) and DIPC1 (126.2 mg, 1.0 mmol) were added thereto in the presence of DMAP (61.1 mg, 0.5 mmol) and allowed to react for 8 hours. The resin was recovered by filtration and washed with NMP and then with methylene chloride, followed by drying under vacuum to give about 1.2 g protected peptide resin wherein the side chain of 3rd serine was octanoylated. To this product 25 was added a de-protecting reagent (10 mL) consisting of 88 % TFA-5 % phenol-2% TIPS-5 % H2O, and the mixture was stirred at room temperature for 2 hours. The resin was removed by filtration, and the filtrate was concentrated followed by adding ether to the resulting residues to form precipitates. The precipitates were recovered by filtration and dried to give about 550 mg crude peptide. 200 mg of this product 30 was dissolved in 10 mL water and applied to YMC-Pack PROTEIN-RP (C4, 20 mmx250 mm) and eluted with a linear gradient (flow rate: 10 mL/min.) for 60 minutes of from 0 to 54 % acetonitrile in 0.1% trifluoroacetic acid. The desired fractions were collected and lyophilized to give about 120 mg of the desired product.

35

**Example of synthesis of a derivative having acyl serine (Fmoc method, car-**

**boxyl-terminal amide compounds)**

Ghrelin (1-9)-NH<sub>2</sub>; GSS(CO-C<sub>7</sub>H<sub>15</sub>)FLSPEH-NH<sub>2</sub>

5 Fmoc-amide-resin (403 mg, 0.25 mmol, ABI Co.,Ltd) was treated with 20% piperazine for 20 minutes and subjected repeatedly to introduction of Fmoc-amino acid by HBTU/HOBt and elimination of Fmoc by piperazine sequentially to construct Fmoc-Ser(Bu)-Ser(Trt)-Phe-Leu-Ser(Bu)-Pro-Glu(OBu)-His(Boc)-resin. After Boc-Gly was finally introduced by DCC/HOBt, the resulting protected peptide resin (about 550  
10 mg) was treated with 1 % TFA-5 % TIPS-methylene chloride solution (10 mL) for 30 minutes. The peptide resin was recovered by filtration, washed several times with methylene chloride (30 mL), and washed with 5 % DIEA (10 mL) and then with methylene chloride (30 mL). The resulting de-Trt peptide resin (about 750 mg) was swollen with NMP (10 mL), and octanoic acid (144.2 mg, 1.0 mmol) and DIPC1  
15 (126.2 mg, 1 mmol) were added thereto in the presence of DMAP (61.1 mg, 0.5 mmol) and allowed to react for 4 hours. The resin was recovered by filtration and washed with NMP and then with methylene chloride, followed by drying under vacuum to give about 800 mg protected peptide resin wherein the side chain of 3rd serine was octanoylated. TFA (10 mL) was added to this product and stirred at room  
20 temperature for 30 minutes. The resin was removed by filtration, and the filtrate was concentrated followed by adding ether to the resulting residues to form precipitates. The precipitates were recovered by filtration and dried to give about 250 mg crude peptide. About 200 mg of this product was dissolved in 10 mL of 30 % aqueous acetic acid and applied to YMC-Pack PROTEIN-RP (C<sub>4</sub>, 20 mmx250 mm) and  
25 eluted with a linear gradient (flow rate: 10 mL/min.) for 60 minutes of from 0 to 54 % acetonitrile in 0.1% trifluoroacetic acid. The desired fractions were collected and lyophilized to give about 150 mg of the desired product.

**Example of synthesis of a derivative having acyl serine (Boc method)**

30

[Ser3(Propionyl)]-rGhrelin (1-28);  
GSS(CO-CH<sub>2</sub>CH<sub>3</sub>)FLSPEHQKAQQRKESKKPPAKLQPR

35

Protected rat ghrelin resin (4-28) was constructed from Boc-Arg (Tos)-Pam resin (0.75 g, 0.5 mmol) by Boc chemistry, and Boc-Ser (CO-CH<sub>2</sub>CH<sub>3</sub>)-OH, Boc-Ser

(Bzl)-OH and Boc-Gly-OH were condensed with a half (1.4 g) of the resin. The resulting resin, 1.5 g, was treated with a mixture of HF and p-cresol (8.5 mL : 1.5 mL) at 0 DEG C for 1 hour, and the HF was evaporated. Ether was added to the residues, whereby 671 mg crude peptide was obtained. This sample was dissolved in 50% acetic acid (AcOH) and applied to a preparative column YMC-Pack-ODS-A (5  $\mu$  m, 20 mmx250 mm) and eluted at a rate of 10 mL/min. by a gradient of from 0 to 95 % acetonitrile concentration in 0.1% TFA solution for 75 minutes. Those fractions containing the desired product were lyophilized to give 135.8 mg crude peptide. A part (0.5 mg) of this product was applied to YMC-A-302 column (C18, 4.6 mmx150 mm) and eluted at a flow rate of 1 mL/min. by a gradient of from 15 to 19% concentration acetonitrile. This purification procedure was repeated and the desired fractions were combined to give 0.41 mg of the desired product.

Other compounds according to the invention may be produced likewise.

#### Example 4

A randomised, single centre, four-period cross-over trial to investigate the absolute bioavailability of iv administered Ghrelin and sc administered Ghrelin at three different single doses in healthy subjects.

#### Objectives:

*Primary:* To investigate the absolute bioavailability of three different doses of Ghrelin administered as single iv and sc doses.

*Secondary:* 1) To investigate the dose linearity (dose proportionality) of the ascending doses. 2) To investigate and compare the pharmacodynamic profiles between the treatments. 3) To assess the safety and local tolerability.

#### Trial Design:

A randomised, single centre, unbalanced block design, four-period cross-over trial to investigate the absolute bioavailability between iv administered Ghrelin and sc administered Ghrelin at three different single doses in healthy subjects. Three doses will be used for each way of administration: low, medium and high. To reduce the number of dosings to each individual and hence reduce the length of the trial each



subject will only receive four doses of the total of six doses, ie. two dose levels administered as iv and sc, respectively. The unbalanced block design will ensure that all three-dose levels will be covered in this way although not all subjects will receive all dose levels. A sufficient washout period will be placed between the individual dosing periods.

**Endpoints:**

Pharmacokinetics of Ghrelin:

AUC<sub>0-t</sub>, AUC, C<sub>max</sub>, t<sub>max</sub>, t<sub>1/2</sub>, Cl/f, Vz/f, Cl, Vz, , t<sub>1/2</sub>, MRT

Pharmacodynamics:

GH: AUC, Cmax and tmax

Cardiac output, assessment of hunger, food/energy intake, degree of pleasure related to food intake, body mass, energy expenditure, DEXA.

**Safety:**

Safety and local tolerability will be assessed throughout the study by clinical evaluations (physical examination and vital signs), electrocardiography and laboratory tests (hematology and clinical chemistry).

**Trial population and power calculation:**

Healthy male subjects, aged 18-45 years with a body mass index (BMI) of 19-26 kg/m<sup>2</sup> (both inclusive).

The primary objective of this study is to investigate the absolute bioavailability of ghrelin administered as iv and sc. An unbalanced block design will be applied to reduce the trial period time and reduce the number of dosings per subject. The number of subjects needed to perform a statistical analysis of absolute bioavailability per dose levels as well as an analysis of dose linearity between doses will be calculated based on existing literature data.

**Trial products:**

Ghelin for iv and sc administration.

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SEQUENCE LISTING

5      <110>    Gastrotech ApS

10      <120>    Use of a secretagogue

15      <130>    P 800 DK00

         <160>    3

20      <170>    PatentIn version 3.1

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20 25

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P 800 DK00

53

1 5 10 15

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20 25

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10 <211> 28

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15 <213> Rattus rattus

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25 <223> Amino acid in position 3 is modified with a fatty acid

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30 Gly Ser Ser Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys  
1 5 10 15

35 Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro Arg  
20 25

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## Claims:

1. Use of a secretagogue compound for the preparation of a medicament for the prophylaxis or treatment of lipodystrophy in an individual in need of such treatment.
2. The use according to claim 1, wherein the secretagogue is ghrelin or a pharmaceutically acceptable salt thereof.
3. The use according to claim 1 or 2, wherein the secretagogue is a ghrelin-like compound or a pharmaceutically acceptable salt thereof

wherein the ghrelin-like compound comprises a structure defined by formula I

$Z^1 - (X^1)_m - (X^2) - (X^3)_n - Z^2$ , wherein

$Z^1$  is an optionally present protecting group

each  $X^1$  is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

$X^2$  is any amino acid selected from naturally occurring and synthetic occurring amino acids, said amino acid being modified with a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

each  $X^3$  is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

wherein one or more of  $X^1$  and  $X^3$  optionally may be modified by a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

$Z^2$  is an optionally present protecting group,

$m$  is an integer in the range of from 1-10

n is 0 or an integer in the range of from 1-35.

4. Use according to any of the preceding claims, wherein m is an integer in the range of from 1-9, such as of from 1-8, such as of from 1-7, such as of from 1-6, such as of from 1-5, such as of from 1-4, such as of from 1-3, such as of from 1-2, such as 2,

5. Use according to any of the preceding claims, wherein  $X^2$  is selected from the group of modified Ser, modified Cys and modified Lys, such as wherein  $X^2$  is modified Ser.

6. Use according to any of the preceding claims, wherein the ghrelin-like compound is selected from a compound of

formula II  $Z^1 - \text{Gly} - (X^1)_{m-1} - (X^2) - (X^3)_n - Z^2$ ,

formula III  $Z^1 - \text{Gly} - \text{Ser} - (X^2) - (X^3)_n - Z^2$ , and

formula IV  $Z^1 - \text{Gly} - (X^2) - (X^3)_n - Z^2$ .

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7. Use according to claim 4, wherein the ghrelin-like compound is having formula III.

8. Use according to any of the preceding claims, wherein  $(X^3)_n$  comprises a sequence selected from one or more of the sequences shown below:

25

Phe Leu Ser Pro Glu His Gln

Phe Leu Ser Pro Glu His

30

Phe Leu Ser Pro Glu

Phe Leu Ser Pro

35

Phe Leu Ser



Phe Leu

Phe

5

9. Use according to any of the preceding claims, wherein n is an integer in the range of from 1-25, such as of from 1-24, such as from 1-15, such as of from 1-10, such as of from 10-25, such as of from 10-24, such as of from 15-25, such as of from 15-24,

10

10. Use according to any of the preceding claims, wherein  $(X^3)_n$  is selected from one or more of the sequences shown below:

15

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Ala Lys Leu Gln Pro Arg

20

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Ala Lys Leu Gln Pro

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Ala Lys Leu Gln

25

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Ala Lys Leu

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Ala Lys

30

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro  
Ala

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro

35

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys

5 Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu

10 Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln

15 Phe Leu Ser Pro Glu His Gln Arg Val Gln

Phe Leu Ser Pro Glu His Gln Arg Val

20 Phe Leu Ser Pro Glu His Gln Arg

Phe Leu Ser Pro Glu His Gln

Phe Leu Ser Pro Glu His

25 Phe Leu Ser Pro Glu

Phe Leu Ser Pro

30 Phe Leu Ser

Phe Leu

Phe

35

11. Use according to any of the preceding claims, wherein the acyl group is selected from a C1-C35 acyl group, such as a C1 – C20 acyl group, such as a C1 – C15 acyl group, such as a C6 – C15 acyl group, such as a C6 – C12 acyl group, such as a C8 – C12 acyl group.
12. Use according to any of the preceding claims, wherein the acyl group is selected from the group of C7 acyl group, C8 acyl group, C9 acyl group, C10 acyl group, C11 acyl group, and C12 acyl group.
13. Use according to any of the preceding claims, wherein the acyl group is selected from the group of C8 acyl group, and C10 acyl group.
14. Use according to any of the preceding claims, wherein the acyl group is selected from the group of C7 acyl group, C9 acyl group, and C11 acyl group, such as from the group of C9 acyl group and C11 acyl group.
15. Use according to any of the preceding claims, wherein the medicament is in a formulation for subcutaneous administration.
16. Use according to claim 15, wherein the formulation comprises the ghrelin-like compound or a pharmaceutically acceptable salt thereof.
17. Use according to any of the preceding claims 14 or 15, wherein the formulation comprises the ghrelin-like compound or a salt thereof as a lyophilisate and the formulation further comprises a solvent, said lyophilisate and said solvent being in separate compartments until administration.
18. Use according to any of the preceding claims 12 or 13, wherein the formulation is a solution of the ghrelin-like compound or a salt thereof.
19. Use according to claim 17 or 18, wherein the solvent is saline.
20. Use according to any of the preceding claims, wherein the medicament is administered prior to or during a meal.

21. Use according to any of the preceding claims, wherein the medicament is administered in a concentration equivalent to from 10 ng to 10 mg ghrelin per kg bodyweight.

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22. Use according to claim 21, wherein the medicament is administered in a concentration equivalent to from 0.1 µg to 1 mg ghrelin per kg bodyweight, such as from 0.5 µg to 0.5 mg ghrelin per kg bodyweight, such as from 1.0 µg to 0.1 mg ghrelin per kg bodyweight, such as from 1.0 µg to 50 µg ghrelin per kg bodyweight, such as from 1.0 µg to 10 µg ghrelin per kg bodyweight.

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23. Use according to any of the preceding claims, wherein the medicament is administered as a bolus prior to or during a meal, said bolus comprising an amount of the ghrelin-like compound or a salt thereof equivalent to from 0.3 µg to 600 mg ghrelin.

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24. Use according to claim 23, wherein the medicament is administered as a bolus prior to or during a meal, said bolus comprising an amount of the ghrelin-like compound or a salt thereof equivalent to from 2.0 µg to 200 mg ghrelin, such as from 5.0 µg to 100 mg ghrelin, such as from 10 µg to 50 mg ghrelin, such as from 10 µg to 5 mg ghrelin, such as from 10 µg to 1.0 mg ghrelin.

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25. Use according to any of the preceding claims, wherein the medicament is administered from one to three times daily, each administration being during a meal or at the most 45 minutes prior to a meal, such as at the most 30 minutes prior to a meal, such as at the most 25 minutes prior to a meal, such as at the most 20 minutes prior to a meal, such as at the most 15 minutes prior to a meal, such as at the most 10 minutes prior to a meal, such as at the most 5 minutes prior to a meal.

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26. Use according to claim 25, wherein the medicament is administered three times daily.

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